Juanita Creek Phase III
Sampling and Analysis Plan

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King County

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Submitted by

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STUDY AREA

Monitoring in 2012 builds upon the intensive bacterial source tracking study completed in 2008 and is part of the continuing efforts of Ecology, King County and the City of Kirkland to identify areas of fecal contamination in the creek. The 2012 efforts will focus on the North Fork tributary to the main stem of Juanita Creek.

Typically, bacterial loadings to urban creeks are highest during the winter rainy season when bacteria from a variety of sources including pets and wildlife can be washed into creeks during storms. Loadings in the summer are lower because of the lack of stormwater/surface flow and because flow in the creek is mostly from groundwater, which is generally of higher quality. However, the bacteria loading to Juanita Creek is also high during the summer low flow. This suggests non-storm driven sources such as leaking sewer pipes, septic systems, or cross connections could be causing these bacteria loadings. As these potential sources are human, exposure to pathogenic diseases is a much more serious public health concern in Juanita Creek and Beach.

DATA QUALITY OBJECTIVES

Accuracy of measurements can be assessed by evaluating both precision and bias. Precision is a measure of data scatter due to random error, while bias is a measure of differences between a parameter value and the true value due to systematic errors. Procedures used to evaluate the precision and bias of sample collection, field measurements and lab analysis are documented in the KCEL Standard Operating Procedures (SOPs) and Quality Assurance Manual. QA/QC for bacterial parameters to be reported for this project are summarized in the Quality Control section. It is expected that the quality objectives for this project will be achieved if the sampling plan and procedures in this document are followed and the frequency and acceptance limits in the Quality Control section are met.

STUDY DESIGN

This study is designed primarily to sample fecal coliform bacteria in Juanita Creek. The King County Microbiology Laboratory is adopting methods for microbial source tracking (MST) using the indicators *Bifidobacteria*, and *Bacteroides*. This study will utilize these new MST methods to provide supplemental information on the usefulness of these types of indicators in identifying the presence of human-source sewage in surface waters. Samples will be collected for all three parameters, fecal coliform bacteria, *Bifidobacteria* and *Bacteriodes*. *Bacteroides* samples will be preserved for later analysis.

Sampling will be at a density sufficient to locate sources so they can be addressed. Also, using several indicator tests will allow the assessment of multiple lines of evidence to evaluate sources. The selection of sampling locations has been designed to isolate tributaries, stormwater pipes, and short stream segments in order to track potential bacterial sources to as small a geographic area as practicable.

Each sampling event will take place over three consecutive days. Data will be collected to provide an estimate of temporal variability by sampling each location twice each sampling day; the first round of samples collected beginning at 9 AM, and a second round of samples collected beginning at approximately 2 PM. We are attempting to collect an estimate of variability both daily (by multiple sampling) and between days. This should provide a more representative picture of the site. If stream segments are identified that indicate a source of bacteria exists between the upstream and downstream sampling location additional efforts will be conducted to specifically locate the source of the bacterial pollution. Visual surveys of the identified stream segment with an analysis of storm drains, septic fields, and sanitary sewer lines will be carried out to locate sources and begin corrective actions to eliminate the bacterial source.

The goals of this study are to investigate and identify reaches of concern along Juanita Creek through periodic intensive synoptic fecal coliform samplings during summer low flow conditions. Additional methods for identifying microbial source tracking indicators will be evaluated. Investigations will also involve streamwalks and windshield surveys to document potential sources. If identified sources warrant immediate required actions (e.g., sewer line breaks), then relevant partners, under their legal authority, will respond accordingly.

Organization and Schedule

Table 1. Project Organization

Name	Title	Affiliation	Phone	Responsibility
Debra Bouchard	Senior Water Quality Planner	WLRD Science	206-296-8252	Project Manager, SAP Preparation
Colin Elliott	Lab Project Manager	KCEL/Lab Project Management	206-684-2343	Lab Project Manager
Eric Thompson	Microbiology Supervisor	KCEL/Microbiology	206-684-2340	Microbiology
Jenny Gaus	Surface Water Engineer Supervisor	City of Kirkland	425-587-3850	Project Manager, Field Coordinator

Kirkland has provided engineering drawings of stormdrain infrastructure in the Juanita Creek drainage basin, map and location of access points. None of the sites are accessed through private property. Sampling Event Dates:

- July 30, July 31, and Aug 1.
- August 20, 21, and 22.

Sampling Locations

Samples will be collected twice daily beginning at 8 AM and a second round of sample collection will begin at 12 PM. This scheduling will allow all samples to be processed at KCEL within the 6 hour holding time for *Bifidobacteri*a samples. Each location will be reconnoitered prior to the commencement of the study to maximize the number of accessible locations that can be safely sampled within the holding time criteria of the *Bifidobacteria* samples.

Table 2. Sampling Station Location and Coordinates

N	Locator	Location	X	Y
1	JUAN111E	1 blk up NE 143rd, downstream of splitter	1305266	269219
2	JUAN111F	On 109th, upstream of where NEJUAN145 connects	1305326	270032
3	NFJUAN145	Near 145th and Oskam's Corner (Ecology site)	1305249	270195
4	NFJUAN145A	Downstream of NFJUAN145	1305168	270015
5	JUAN112	Above Juanita Woodinville Rd, near 112th (Ecology site)	1306204	272028.
6	0446-58	Below culvert in ditch SE of Juanita-Woodinville Rd and south of NE 149th St.	1305992	271508

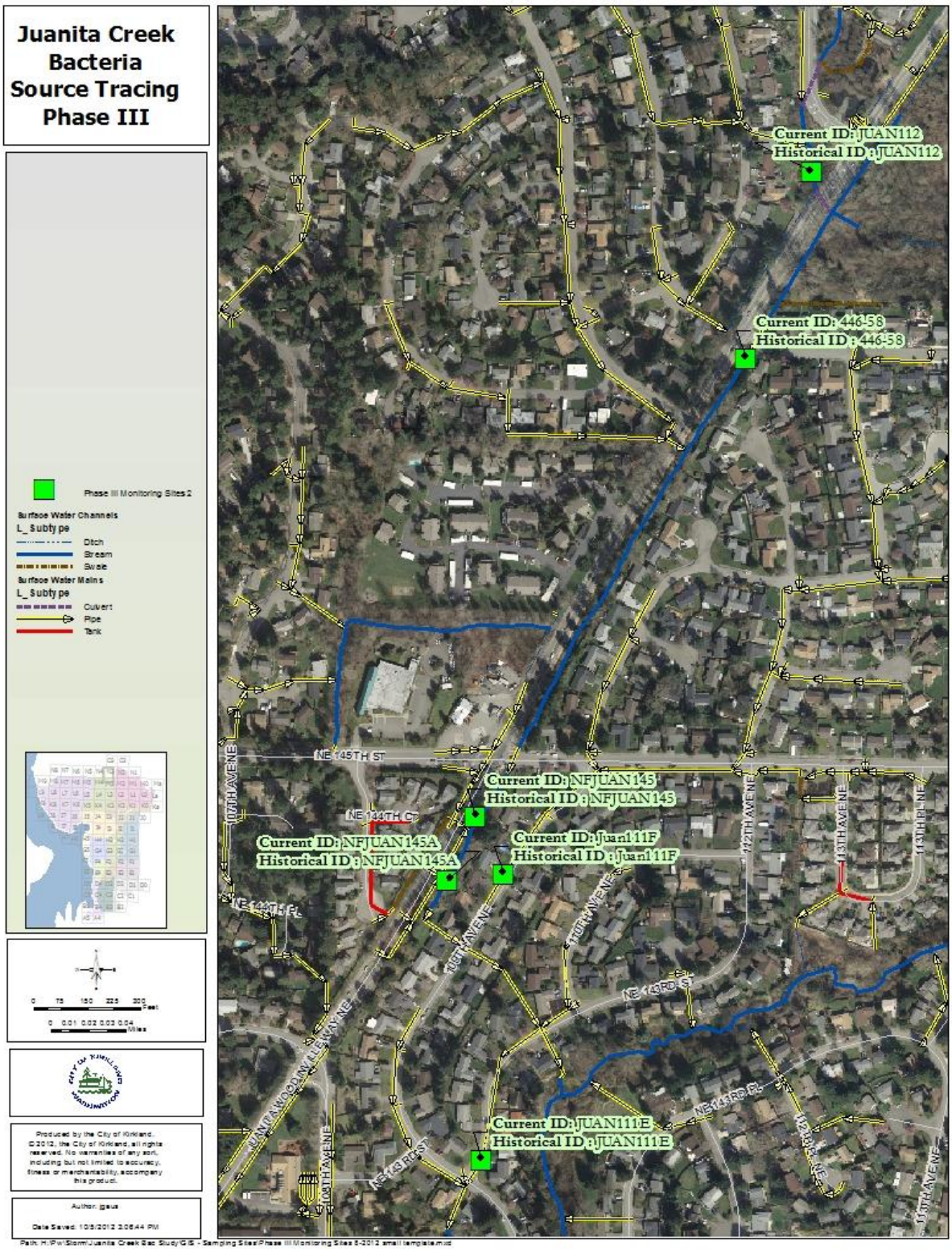

Figure 1. Samping Locations.

Sampling Procedures

Grab samples for bacteria testing and source evaluation will be collected at each sampling location for every designated sampling run. If the selected stream segment is inaccessible from the streambank, a sampling pole with a 1L bottle holder will be used to collect the grab sample. If the selected stream segment is dry, samples will not be collected, and lack of flow will be noted on the field sheets. A digital photograph will be taken at each sampling location during the initial site visit, focusing on the wetted portion of the stream where the sample is collected. Field observations will be recorded by sampling personnel if significant changes in stream flow or other factors are observed that may impact data quality.

1) When possible, sample collection will begin at the downstream locations and proceed upstream to avoid any problems with sediment disruption. Prior to entering the stream, the sampler determines the safety of entry and if deemed safe, enters just downstream of sample site, wading in a manner to avoid disturbing the water with sediment disruption. If the sampling site cannot be reached safely by foot, a sampling pole with a bottle holder will be used to collect the grab sample.

2) Samples should be collected from the deepest, swiftest moving portion of the stream, especially during low flows.

3) Containers must not be pre-rinsed with sample prior to collection.

4) Using the sterile bottle that does not have preservative, the sampler (facing upstream) removes the cap from the sample bottle, tips the sample container downward at a 45 degree angle and plunges the container so that the mouth is approximately 5 inches below the surface. In the same motion, the sample container is turned upward so it begins filling with ambient water. The container must remain below the surface until filled to the shoulder. Transfer the water from the collection bottle to the sterile bottle containing the preservative sodium sulfite. Finish this transfer slowly so that there is no spill over that will flush out the preservative. Leave NO headspace.

5) Use the same collection bottle and technique to collect the water for fecal coliform and *Bacteriodes* analysis. This time leave a 1-inch headspace before capping the container.

6) Filled containers should be stored immediately in ice-filled coolers during transport to the lab. Equipment decontamination for bacterial samples is not necessary since all samples will be collected directly into the lab container. Each container has been sterilized prior to delivery to the field.

7) Record sample time on both the bottle and the field sheet.

Table 3. Sample Containers and Preservation

Parameter	Container Type	Field Preservation
Fecal Coliforms, *Bacteroides*	500 ml Polypropylene, Sterile	Store on ice
Bifidobacteria	500 ml Polypropylene, Sterile	Sodium sulfite No headspace Store on ice

Container labels will include:

- Lab Sample Number
- Sample Location ID (Locator)
- Date and Time of Collection
- Parameter

LABORATORY METHODS AND QUALITY CONTROL

No Field measurements (Temp, DO, pH, cond) will be collected for this study.

Analytical Methods and Detection Limits

Samples will be collected for fecal coliform, *Bifidobacteria*, and *Bacteroides* spp. by q-PCR. All analyses will be done at the KCEL.

Table 4. Methods and Detection Limits

Parameter	Reference Method and Technique	Reported Units	Lower Reporting Limit	Holding Time	Preservation
Fecal Coliform	Standard Methods, 9222D Membrane Filter	cfu/100 mL	1 cfu/100 mL	24 hours	Cool to <10ºC
Sorbitol – fermenting Bifidobacteria	University of Wisconsin Communication with Dr. Sharon Long KCEL SOP	Cfu/100 ml		3–6 hours	Cool to <10ºC , no headspace
Bacteroides (q-PCR)	Converse, R.R. et al, 2009 Griffith, J.F. et al, 2009 KCEL Draft SOP	cells/100 ml	1 cell/100 ml	24 hours	Cool to <10ºC Freeze filters for storage

Quality Assurance and Quality Control

Lab Measurements: Routine QC analyses for all bacterial tests monitor method performance of each sample analysis batch. A sample analysis batch should not exceed 20 samples of the same matrix which are all prepared together and analyzed using the same reagents, media equipment, and by the same analyst(s). The QC samples to be tested with this set of samples are described below:

Laboratory duplicates are prepared for each matrix type at a frequency of 1 per batch or 5%, whichever is more frequent. The duplicate must be processed through all preparation and incubation steps used for the original sample. The acceptance limits are based on a 95% confidence limit as described in the appropriate reference method.

A negative control is prepared at a frequency of 1 per batch or 5%, whichever is more frequent. The negative control should show an appropriate qualitative response for the test organism and should not be identified as containing the target organism.

	Negative Control
Fecal Coliforms	*Proteus sp. or* *Enterobacter sp.*
Bifidobacteria sp.	*Bifidobacteria breve ATCC 15698*

A positive control is prepared at a frequency of 1 per batch or 5%, whichever is more frequent. The positive control should show an appropriate qualitative response for the test organism.

	Positive Control
Fecal Coliforms	*E.coli*
Bifidobacteria sp.	*Bifidobacterium breve ATCC 15700*

For Bacteroides testing, positive controls (template/target controls) negative controls (non-template/non-target controls) and calibrator samples will be included in each instrument run.

Pre-filtration and post-filtration blanks are prepared each working day to evaluate the sterility of the dilution water and filtration equipment. These sterility controls are considered acceptable if no growth is detected.

The focus of this survey is to identify potential bacterial sources to short segments of stream, and once located, to initiate corrective actions. While the design has inherent variability issues, it is assumed that with sequential sampling upstream any contributors to the bacterial count variability will be similar at adjacent sites and that the absolute difference will not be significantly influenced local variability. This study is neither a loading study nor a TMDL and *quantification of the absolute counts* is secondary to the absolute differences between adjacent sampling locations.

In order to give an indication of how consistent and reproducible laboratory methods are a measure of precision is calculated. KCEL estimates precision by calculating the Relative Percent Difference (RPD) of the duplicate sample results:

Approximately 5% of the laboratory samples will be analyzed in duplicate to provide a means of assessing *analytical* precision.

$$RPD = \frac{|X_1 - X_2|}{(X_1 - X_2)/2} 100$$

Analytical precision is determined by performing a duplicate analysis on the same sample and comparing the results. Laboratory duplicates by the membrane filtration method are performed by removing aliquots from the sample bottle as two separate sub-samples, and duplicating all steps including preparation of dilutions. Duplicate sample results are evaluated by method 9020B.4 prescribed in Standard Methods for the Examination of Water and Wastewater, 20th ed., 1998. Briefly, this requires that the log-transformed difference between the two duplicate results be compared to the mean of the log-transformed differences for the previous 15 sample pairs. The acceptance criterion is to be within 3 standard deviations of this latter value. Failure to meet the criterion is cause to evaluate the entire sample batch for compliance and applicability of the calculation, before qualifying or rejecting the data set.

Note, higher variability with low count results is especially noticeable for bacteria. Therefore,) for bacteria parameters duplicate pairs will be divided initially into two categories: (1) those pairs with a mean less than or equal to 20 cfu/100 mL; and (2) those pairs with a mean greater than 20 cfu/100 mL. For the second category, the mean relative standard deviation (*RSD)* of duplicate pairs will be evaluated by a cumulative frequency distribution. The project manager will review duplicate pairs in the first category, as well as sample sets with less than 10 replicate pairs, to determine the usability of the data.

Laboratory Data Delivery

The King County Environmental Laboratory will provide a 45-day turnaround time for all bacterial data starting upon receipt of the last sample collected per event. PCR/Bacteroides testing may take longer since the decision on which samples to test may not be made until all bacterial results are available. Following completion of all analysis, the Laboratory Project Manager will provide a narrative describing the contents of the lab data packages, including any notable information of immediate interest to the recipient.

QA Review

Project data will undergo standard QA review within each laboratory group according to the Environmental Laboratory QA document and method-specific SOPs. Data will be flagged accordingly. A description of the laboratory qualifiers is provided below. Data Anomaly Forms

will be prepared in the event of a significant quality issue with the samples. These will be available for project managers to review.

Data Qualifiers: If it is determined in the review process that the quality objectives were not met or an analysis anomaly has occurred, the affected data will be flagged and the project manager notified. Data qualification flags, which may be entered to LIMS, are presented in the table below:

Table 5. Data Qualifiers

Qualifier	Description
H	Indicates that a sample handling criterion was not met in some manner prior to analysis. The sample may have been compromised during the sampling procedure or may not comply with holding times, storage conditions, or preservation requirements. The qualifier will be applied to applicable analyses for a sample.
R	Indicates that the data are judged unusable by the data reviewer. The qualifier is applied based on the professional judgment of the data reviewer rather than any specific set of QC parameters and is applied when the reviewer feels that the data may not or will not provide any useful information to the data user. This qualifier may or may not be analyte-specific.
J	Indicates the reported value is an estimated value.
TA	Applied to a sample result when additional narrative information is available in the text field. The additional information may help to qualify the sample result but is not necessarily covered by any of the standard qualifiers.
C	Applied to fecal coliform data when the sample analysis exhibits confluent growth of organisms. The value reported can be reliably used as an indicator of relative abundance; however, it can not be used as an accurate count of the associated organism.
>#####	Applied in to fecal coliform data when the population count exceeds the procedural capacity to measure quantitatively. The number in the qualifier is the highest procedural count or concentration possible for the sample dilutions analyzed. A value is not entered into the numvalue field. The actual population count is at least as great as or greater than the value reported in the qualifier.
Fail	The result if the positive or negative control fails
Pass	The result if the positive or negative control passes

Data Storage: All field and sampling records, custody documents, raw lab data, and summaries and narratives will be archived according to KCEL policy.

Corrective Action Procedures

Individual SOPs describe specific corrective action for each analytical procedure and quality control measure. If QC samples exceed their control limits, the analysis is repeated if possible, or documented and affected samples qualified. If samples are lost or compromised, the project manager must determine whether to re-sample or to disregard the specific parameter or event.

Documentation/Record Keeping

Within the analytical laboratory, each section and analytical procedure has its own documentation protocol. The minimum documentation required in the lab includes an instrument logbook, analysis log, calibration and analysis documentation and LIMS hardcopy sheets.

For all analytical results generated by lab activities, sufficient hardcopy data must be stored such that a reviewer could verify that the requirements of the reference method and SOP were met. The format of stored data may include logbook entries, field notes, bench sheets and printouts of instrument or data files. Storage of only the electronic version of these documents is not sufficient to meet current data storage requirements.

Data Reporting

Data package will include King County Environmental Laboratory Comprehensive Reports that include all project parameters, microbiology narrative data including supporting QC documentation and a technical memorandum, summarizing field sampling, analytical work, and interpretation of the QC results (provided by the King County Environmental Laboratory).

Data Records

Hand written information used as supporting documentation, which is not stored directly with the analysis results, such as standards preparation records and equipment calibration checks, must be maintained in logbooks. Data packages are peer reviewed and stored in filing cabinets. Laboratory bench worksheets should e written using indelible black ink (no pencils) and dated and initialed. Individual data bench worksheets must be uniquely identified if they are to be referenced in other documents. All deletions and corrections must be a single line cross-out, accompanied with the date and initials of the person making the correction.

Storage of Lab Data

Procedures for the storage and disposal of hardcopy lab data are summarized in King County Environmental Lab's SOP # 11-01-005-001 (Records Storage) which is based on King County and Washington State governmental records storage requirements. It is the policy of the lab to store all data packages, supporting documentation and project records for a minimum of 10 years, based on the date of sample collection or field data measurement.

In LIMS, final sample and QC data is maintained indefinitely in the EDS database, which is backed up daily. Additional LIMS information specific to sample management is maintained a minimum of 1 year past the date the final results were posted. Other types of electronic data such as instrument files and photographs may be stored but no lab-wide policy is currently available.

Late Check-in

Samples are collected by City of Kirkland personnel. Currently, this project is scheduled to be completed during routine business hours with sample delivery to occur by 4:00 p.m. If sample delivery is delayed for any reason, field crew will contact the KCEL Sample Drop Off (Lynn Cox) 206-684-2390 or the KCEL main desk at 206-684-2300.

REFERENCES

Stoeckel and Harwood. 2007. Performance, design, and analysis in microbial source tracking studies. Applied and Environmental Microbiology p. 2405-2415.

Standard Methods, 21st Edition